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RAPID PUBLICATION

WEILL-MARCHESANI SYNDROME - POSSIBLE LINKAGE OF THE AUTOSOMAL DOMINANT FORM TO 15q21.1

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Weill-Marchesani syndrome comprises short stature, brachydactyly, microspherophakia, glaucoma, and ectopia lentis is regarded as an autosomal recessive trait (McKusick 277600). We present two families each with affected individuals in 3 generations demonstrating autosomal dominant inheritance of Weill-Marchesani syndrome. Linkage analysis in these 2 families suggests a gene for Weill-Marchesani syndrome maps to 15q21.1. The dislocated lenses and connective tissue disorder in these families suggests that fibrillin-1 and microfibril-associated protein 1, which both map to 15q21.1, are candidate genes for Weill-Marchesani syndrome. Immunohistochemistry staining of skin sections from family 1 showed an apparent decrease in fibrillin staining compared to control individuals.

KEY WORDS: Weill-Marchesani, fibrillin-1, chromosome 15, linkage

INTRODUCTION

In 1932, Weill, an ophthalmologist from Strasbourg, described a woman with ectopia lentis, short stature and stubby, swollen fingers impeding closure of the fist. Marchesani [1939], then living in Münster, later professor of ophthalmology in Hamburg, reported on two families. The first consisted of an 8-year-old boy, who had all of the aforementioned signs; his parents were described as short with short hands. The second kindred consisted of an affected brother and sister and the parents, who were stated to be short. Since parental consanguinity was demonstrated in one kindred, Marchesani suggested that short stature was the heterozygous expression of this rare "recessive condition". This opinion was supported by Meyer and Holstein [1941], who reported 4 of 7 affected sibs with normal but consanguineous parents. The hypothesis that "spherophakia-brachymorphia" was an autosomal recessive trait was corroborated by Kloepfer and Rosenthal [1955] and Rosenthal and Kloepfer [1956]. Since then, over 100 cases have been reported among the Amish and other groups [see Maumenee, 1993].

However, Gorlin et al. [1974] reported a two-generation family (father, son , daughter) with short stature, brachydactyly with joint stiffness, progressive microspherophakia with severe myopia, glaucoma, and ectopia lentis. Young et al. [1986] noted a mother and

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son with short stature, joint restriction, myopia, lens dislocation, and glaucoma. Verloes et al. [1992] described a 3-generation family with similar findings. They further suggested a possible relationship with Moore-Federman syndrome (taut skin, joint restriction, brachydactyly, hyperopia, late onset glaucoma) [Moore and Federman, 1965; Winter et al., 1989].

Less well-documented examples of autosomal dominant inheritance of Weill-Marchesani syndrome are those of Jensen et al. [1974] (father and daughter); Pietruschka and Priess [1973] (father and 4 sons); and Probert [1953] (father and 4 children).

Here, we extend the pedigree first presented by Gorlin et al. [1974] to include 3 affected generations, and present a new family with autosomal dominant Weill-Marchesani syndrome. Because of the clinical findings, including dislocated lenses, we propose that fibrillin-1 is a candidate gene for Weill-Marchesani syndrome. Finally, we have an indication of linkage of Weill-Marchesani syndrome in both families with chromosome 15 markers including and encompassing FBN1 and abnormal fibrillin-1 immunostaining in skin sections from family 1.

MATERIALS AND METHODS

Clinical Evaluation

History and physical assessment was obtained from all 32 participants in the two families. Measurements were made of height, arm span, lengths of hand and foot according to the methods of Hall et al. [1989]. All participants were evaluated for myopia, glaucoma and dislocated lenses. Blood and/or skin biopsy were obtained, following informed consent and DNA isolated according to standard protocols [Sambrook et al., 1989].

Microsatellite Marker Typing

Microsatellite markers reported by Gyapay et al. [1994] were purchased from Research Genetics and included D15S165, D15S118, D15S214, D15S126 and D15S209. The markers defined by Mts2 [Pereira et al., 1994] and G113 [Hudson et al., 1992] are within FBN1 and were the kind gift of Dr. Bradley Popovich. PCR reactions contained 0.25-0.5 U *Taq* polymerase in 6.25 µl vol containing 10 ng of forward and reverse primers, 0.2 mM of each deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 50 mM KCl, 3 mM $MgCl_2$ and 10 mM Tris-HCl (pH 9.0 at 25°C). Twenty-nine cycles of PCR were performed as follows: one round at 94°C for 5 min followed by 29 cycles (94°C, 10s; 55°C, 10s; and 74°C, 30s) followed by 5 min at 72°C and stored at 4°C. The resulting PCR products were mixed 1:1 with formamide loading buffer [Sambrook et al., 1989] and 2 µl was applied to an 8% denaturing acrylamide gel containing 5.6 M urea and 32% formamide [Litt et al., 1993]. The gel was transferred by capillary blotting [Litt et al., 1993] to a positively charged nylon membrane (Boehringer-Mannheim) and probed with a $(CA)_{15}$ 3' oligomer labeled with digoxigenin-11-dUTP, and detected colorimetrically with the Genius Kit from Boehringer-Mannheim. Amplification reactions were repeated if the blots were not readable. If still ambiguous, the sample was omitted from the analysis. Two individuals scored the gels independently.

Linkage Analysis

We conducted two-point and multipoint linkage analyses using the VITESSE computer package [O'Connell and Weeks, 1995]. VITESSE was particularly useful for this analysis since no recoding of marker alleles is necessary for large runs. We assumed autosomal dominant inheritance of a rare gene (frequency = .0001). Since onset of Weill-Marchesani syndrome occurs early in life, no age correction was required. Because reliable estimates of penetrance are not available from published data, we conducted the linkage analysis in two ways: 1) we assumed a high penetrance of 0.90, based on the pattern of occurrence of the disease in these two families, and 2) we used an "affecteds-only"strategy. Specifically, those individuals with the syndrome were coded as affected, all others were coded as unknown with respect to disease status, and a very low estimate of penetrance (0.01) was specified. Since Weill-Marchesani syndrome is a rare disorder, no phenocopy rate was incorporated into the analysis.

We analyzed two microsatellite markers known to be contained within fibrillin-1, Mts2 and G113, as well as 5 microsatellite markers that span ~28 cM

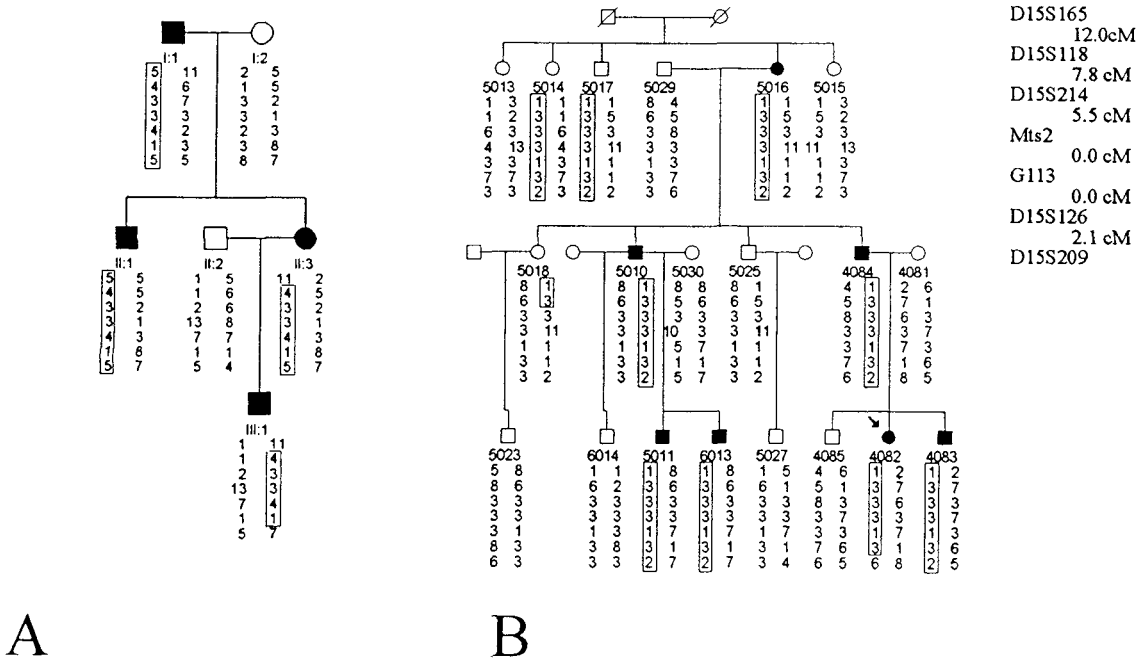

Fig. 1. Pedigrees of families 1 and 2 and haplotypes of chromosome 15q markers. Blackened symbols denote affected individiuals, clear symbols denote unaffected individuals. Genotypes are listed in the order given by the map in the box at the upper right. Samples were available for all individuals for whom a haplotype is shown. The haplotype of the disease-bearing chromosome is boxed. Family 1 is shown in panel A and Family 2, in panel B.

across this region of 15q. The map order and distances for these 5 markers were obtained from Gyapay et al. [1994]. In order to map the fibrillin-1 markers, we obtained two-point linkage data from Généthon [Dib et al., 1996] for fibrillin-1 and each of the other 5 markers in the region. The resulting map is indicated in Fig. 1. Published allele frequencies were available from the World Wide Web [Dib et al., 1996]. These frequencies were comparable to those calculated from 56 control chromosomes from our glaucoma population.

Fibrillin Immunofluorescence

Skin immunostaining was performed as previously described [Godfrey et al., 1990].

RESULTS AND DISCUSSION

Clinical Manifestations

Family 1

In 1994, the family reported by Gorlin et al. [1974] was reexamined because a third generation (a son of the affected daughter) was affected (see Fig. 1A). There was no consanguinity in any of the matings.

Skeletal system. Stature is short and proportionate. Adult male height ranges between 142-157 cm with females 135-140 cm tall. All 4 patients have limited mobility of joints, especially in the hands

Table I. Physical Measurements of Affected Family Members from Family 2.

Pedigree #	Age yrs	Height cM	Span cM	Hand cM	Foot cM	Birth Length inches
5016	60	160.0	147.3	16.5*	21.5	
5010	39	166.4*	167.6	16.5*	21.5*	18*
4084	34	168.9*	167.6	17.3*	24.5*	18*
5011	5	107.3*	106.7	12.1*	14.6*	18.7*
4083	9	137.2	133.4	14.0*	19.8*	19.25
4082	11	145.4	145.4	15.4	21*	20.5
6013	2	81.0*	nm	nm	nm	21.5

*3-10th centile
nm = not measured

where neither full flexion nor extension can be achieved. Motion at the wrist and elbows is also reduced. The fingers and toes are short with knobby joints. The palmar fascia is thickened, the overlying skin appearing puckered. Individual II-3 has carpal tunnel syndrome.

Ocular findings. Myopia, microspherophakia, iridodonesis, dislocated lenses and shallow anterior chambers occur in all affected. The lenses may be dislocated at birth (as in the case of our new patient, III-1) or may dislocate as late as the end of the first decade. Cataracts are a frequent complication. The myopia is severe, often being between 10-20 d and glaucoma appears in most cases before puberty. The glaucoma may be acute or manifest as repeated self-limiting attacks.

Family 2

The index case (Fig. 1B, individual 4082), was first seen for evaluation of visual impairment at age 7. She was found to have microspherophakia, glaucoma, brachydactyly, and myxedema-like thickening of her skin. Five other relatives were found to be similarly affected as shown in the pedigree (Fig. 1B). The index case's paternal grandmother, 5016, was the first affected relative; by history, her parents were of normal stature and had no visual problems, and her 7 sibs, 4 of whom we examined, were unaffected.

Skeletal system. The proposita's paternal grandmother, father and uncle all were short of stature and had glaucoma. All affected relatives were at or below the 25th centile for hand length and foot length, but had normal upper to lower segment and span to height ratios (Table I). These parameters were in the 50-90th centile for unaffected relatives. Also, all of the affected relatives except individuals 4082 and 4083 were below the 25th centile for height. Individuals 4082 and 4083 were at the 25-50th and 50-75th centile, respectively. Considering that their mother, paternal aunt and uncle were all above the 90th centile in height, the above two individuals' heights are short in comparison to family members. Birth length was known for 10 relatives and showed the same contrast, i.e., less than 25th centile for 3 affected and 75-90th centile for all unaffected relatives. The thickened skin was also present in all affected individuals. Joint mobility was unimpaired in both affected and unaffected relatives.

Ocular findings. The grandmother (Fig. 1B, individual 5016) had glaucoma and was aphakic in OD. Her left eye had been removed in her late 30's because it was blind and painful; in addition, her fingers were short and stiff. Her son (individual 4084) received glasses at age 3 and developed glaucoma at 9. He had undergone bilateral lensectomy and vitrectomy and had sustained postoperative retinal detachments OU. Both he and his brother (individual 5010) had carpal tunnel syndrome. This brother also had dislocated lenses and glaucoma in early childhood.

TABLE II. Two-Point Lod Scores for Weill-Marchesani Syndrome With Chromosome 15 Markers.

Marker	θ=					
	0.00	0.01	0.05	0.10	0.20	0.30
D15S165 penetrance = .9	-2.15	0.08	0.67	0.83	0.83	0.65
affected individuals only	-1.89	0.08	0.67	0.83	0.83	0.65
D15S118 penetrance = .9	2.11	2.07	1.91	1.71	1.29	0.86
affected individuals only	2.11	2.07	1.91	1.70	1.29	0.86
D15S214 penetrance = .9	1.20	1.18	1.09	0.98	0.75	0.50
affected individuals only	1.20	1.18	1.09	0.98	0.75	0.50
Mts2 penetrance = .9	0.60	0.59	0.54	0.47	0.34	0.21
affected individuals only	0.60	0.59	0.54	0.47	0.34	0.21
G113 penetrance = .9	1.20	1.18	1.09	0.98	0.75	0.50
affected individuals only	1.20	1.18	1.09	0.98	0.75	0.50
D15S126 penetrance = .9	1.50	1.47	1.35	1.19	0.88	0.57
affected individuals only	1.54	1.47	1.35	1.19	0.88	0.57
D15S209 penetrance = .9	-6.50	-2.50	-1.16	-0.63	-0.18	-0.01
affected individuals only	-5.50	-2.48	-1.16	-0.63	-0.18	-0.01

The index case (individual 4082, Fig. 1B) had consistent intraocular pressures in the low-to-mid-20's with pressures being 28 mm Hg on the OD and 26 mm Hg on the OS in 1991 at the time of presentation. Goldmann visual fields of the proposita have always been normal. Her vertical cup-to-disc ratios are in the range of 0.5. The proposita's brother (individual 4083, Fig. 1B) had optic nerves with vertical cup-to-disc ratios in the range of 0.4 and intraocular pressures which were elevated at 24 mm Hg in the right eye and 25 mm Hg in the left eye using a tonopen-2. With an applanating tonometer the pressures were 24 mm Hg in both eyes. In several years of subsequent follow-up, his intraocular pressure remained in the low-to-mid 20's but at one time was found to be 32 mm Hg OD. The optic nerve OD has shown borderline progression. This family has Weill-Marchesani syndrome with 3 generations affected, showing male-to-male transmission.

Linkage Analyses

Two-point lod scores are reported in Table II, summed for both families. Evidence for linkage with FBN1 is indicated. Neither FBN1 marker, by itself, is very informative: at Mts2 the linked allele in both families ("3") is the most common allele in the population (44%); this is also the case at G113 in family 1. Haplotypes for the 7 markers are shown in Fig. 1. As expected, multipoint analysis provides stronger evidence for linkage with fibrillin in these two families. In the analysis with penetrance of 0.90, a maximum multipoint location score of 2.22 occurred at Mts2/G113/D15S126 (Fig. 2). The "affecteds-only" analysis provided very similar results; however, the maximum multipoint location score, 2.11, occurred across the 13 cM region from D15S118-D15S126. Crossovers occurred in affected individuals at D15S118 (proximal to fibrillin-1) and D15S209

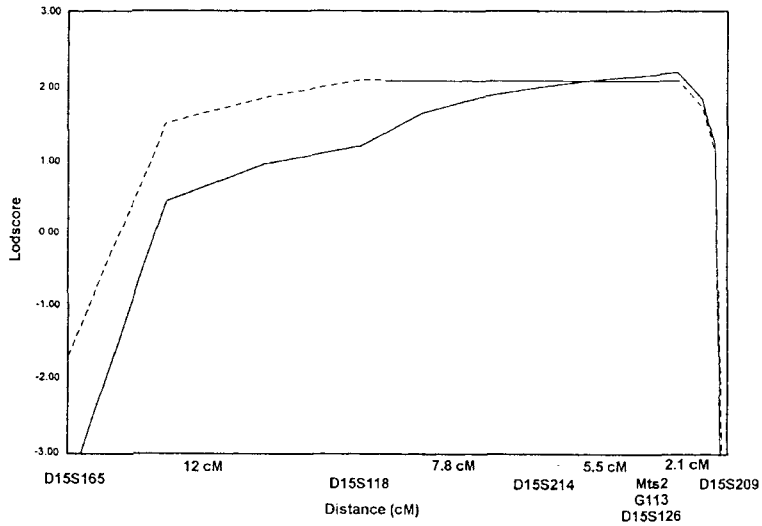

Fig. 2. Multipoint linkage results for the analysis with penetrance estimated at 0.90 (solid line) and "affecteds-only" analysis (dotted line).

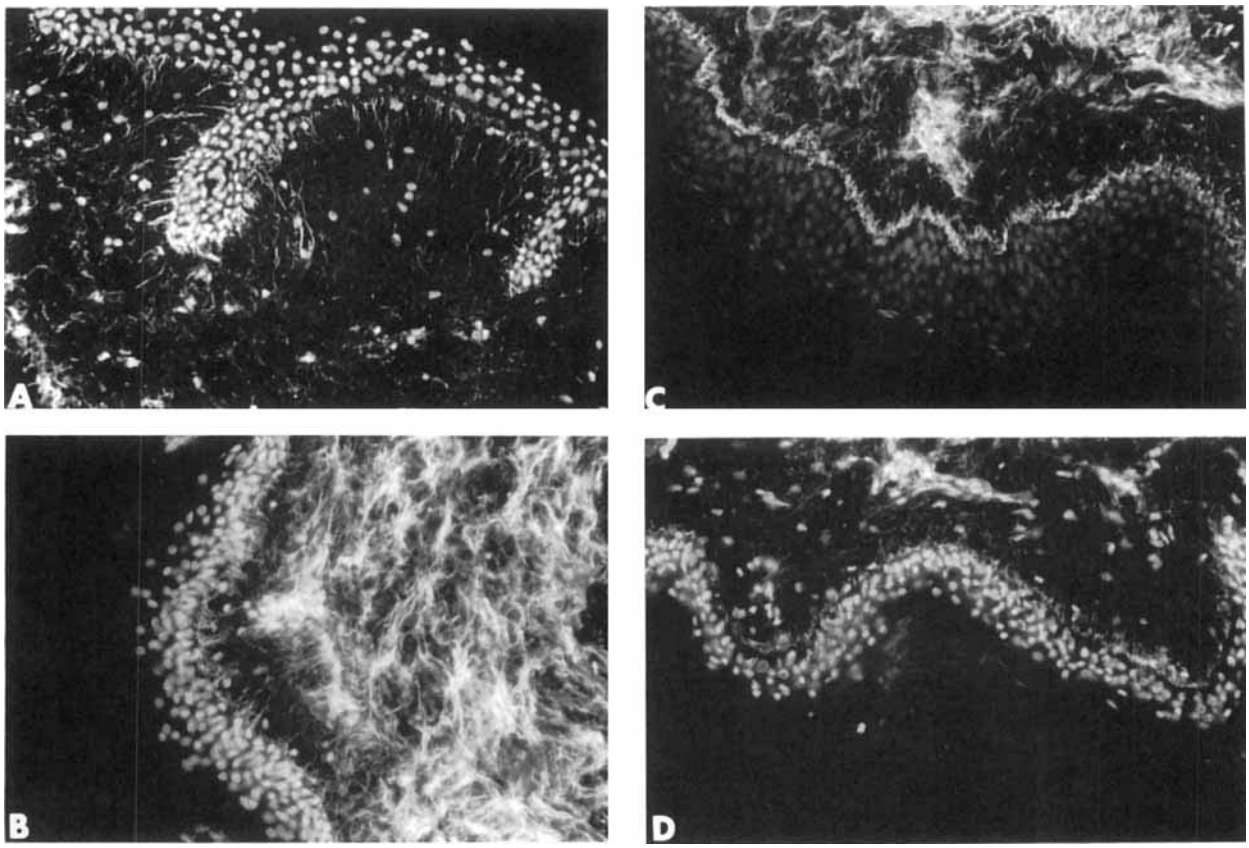

Fig.3. Fibrillin immunostaining of skin sections from individuals in family 1. Panels A, C, and D are from affected individuals I-1, II-1, and II-3, respectively, (see Figure 1A). Panel B is from the unaffected mother (I-2) of individuals (II-1 and II-3). The decrease in immunostaining at the dermal/epidermal junction and in the dermis of individuals I-1, II-1, and II-3 (panels A, C, D) are clearly apparent when compared to individual I-2 (panel B) who is unaffected.

(distal). These data provide evidence that FBN1 is a candidate gene for autosomal dominant Weill-Marchesani syndrome in these two families. (Linkage *analyses in family* 1 have excluded FBN2 [Wang and Godfrey, unpublished]).

In family 2, two of the normal sibs of individual 5016 carry the same haplotype as the affected relatives. There are two possible explanations for this finding. Because both of the parents of this sibship have been reported to be normal, as well as their sibs and parents, we propose that individual 5016 represents a new mutation resulting in Weill-Marchesani syndrome. New mutations are not uncommon in other connective tissue disorders including osteogenesis imperfecta, Marfan syndrome and achondroplasia [Carothers et al., 1986; Vogel and Rathenberg, 1975; Jones et al., 1975; Orioli et al., 1995]. Given that individual 5016 is one of the last in birth order in her sibship, paternal ageing could well have been a primary factor as has been shown in the above diseases. An alternative hypothesis is that there is incomplete penetrance of Marchesani syndrome in the two normal sibs as well as in one of their parents.

Fibrillin Immunohistochemical Staining

Because of analogies to Marfan syndrome, neonatal Marfan syndrome and congenital contractual arachnodactyly, fibrillin immunofluorescence studies were carried out. Fibrillin immunostaining of skin biopsies from affected relatives (I-1, II-1, and II-3) showed a generalized decrease in immunostainable fibers when compared to the unaffected control (I-2) (Fig. 3). Diminished fibrillin immunofluorescence was noted at the dermal-epidermal junction and in the papillary dermis. Interestingly, staining with toluidine blue or observation of elastin autofluorescence in unstained sections showed an apparent increase in elastic fibers in the affected individuals compared with control individuals (data not shown). These findings implicate elastin associated microfibrils in general and, perhaps, fibrillin-1 specifically in the pathogenesis of dominant Weill-Marchesani syndrome (skin sections were unavailable in family 2.)

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